When Every Second Counts
Pathogen Identification in Less than a Minute

WHEN there's anthrax in the air—or indeed, any pathogen—the sooner one knows, the better. Ideally, a detector would identify the pathogen in time to take action, an interval referred to as detect-to-warn, which is generally considered to be a minute or less.

Commercial systems exist that can identify airborne pathogenic spores, but they take days or, at best, hours to produce results. Far too long to hold one's breath.

A system being developed by Lawrence Livermore to identify such spores—the bioaerosol mass spectrometry (BAMS) system—recently broke that critical 1-minute time barrier. Livermore chemist Eric Gard heads up a team developing this mass-spectrometry technique, which can successfully distinguish between two related but very different spore species. It can also sort out a single spore from thousands of other particles—both biological and nonbiological—with no false positives.

The biomedical aspects of this work are funded by the Laboratory Directed Research and Development (LDRD) at Livermore, and the biodefense aspects are funded by the Technical Support Working Group and Defense Advanced Research Project Agency of the Department of Defense.

When Time Is of the Essence

The premise of a detect-to-warn system is to allow time to react. "A minute gives people enough time to put on masks, leave the room, hold their breath. The challenge was to actually make a device that could provide answers in less than a minute," explains Gard.

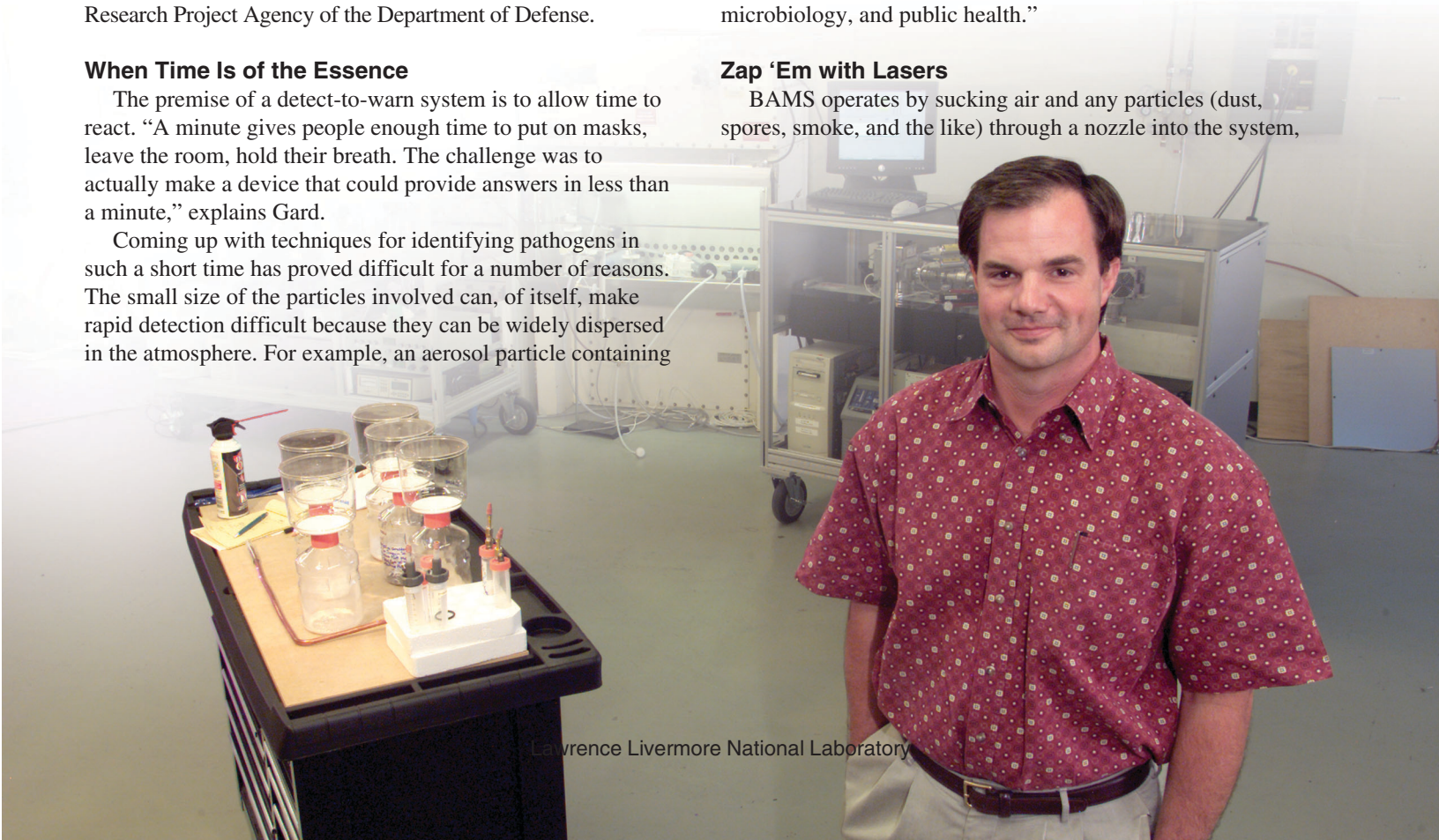
Coming up with techniques for identifying pathogens in such a short time has proved difficult for a number of reasons. The small size of the particles involved can, of itself, make rapid detection difficult because they can be widely dispersed in the atmosphere. For example, an aerosol particle containing a single *Bacillus anthracis* spore has a mass of approximately one-trillionth of a gram. Of the methods available to detect anthrax and other airborne uglies, most take hours, even days, to yield results, making timely actions impossible.

The issue of false alarms is also critical—some techniques have difficulty separating organisms that are benign from those that are pathogenic but very similar. The situation becomes even more complicated because some pathogens, such as smallpox, are highly contagious, requiring just a few organisms to infect a person. The system should ideally be sensitive enough to find and identify a single particle among other naturally occurring background particles, which could be present at concentrations thousands of times higher.

The BAMS technique, which Gard and others have been working on for nearly five years, can successfully identify a single airborne particle in about 100 milliseconds. This technique has other applications as well, Gard notes. "In the future, BAMS could also be used as a medical diagnostic to, for instance, track small subpopulations of cancerous cells that deviate from their normal development cycle. As such, BAMS may make far-reaching contributions in the fields of oncology, microbiology, and public health."

Zap 'Em with Lasers

BAMS operates by sucking air and any particles (dust, spores, smoke, and the like) through a nozzle into the system,



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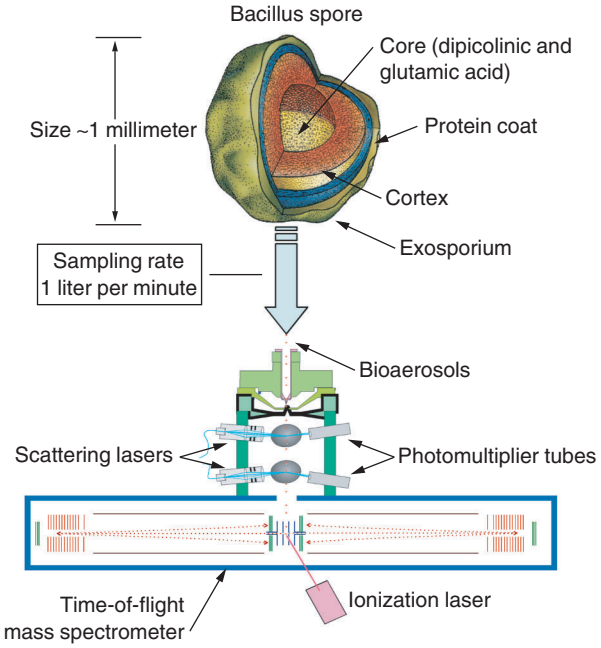
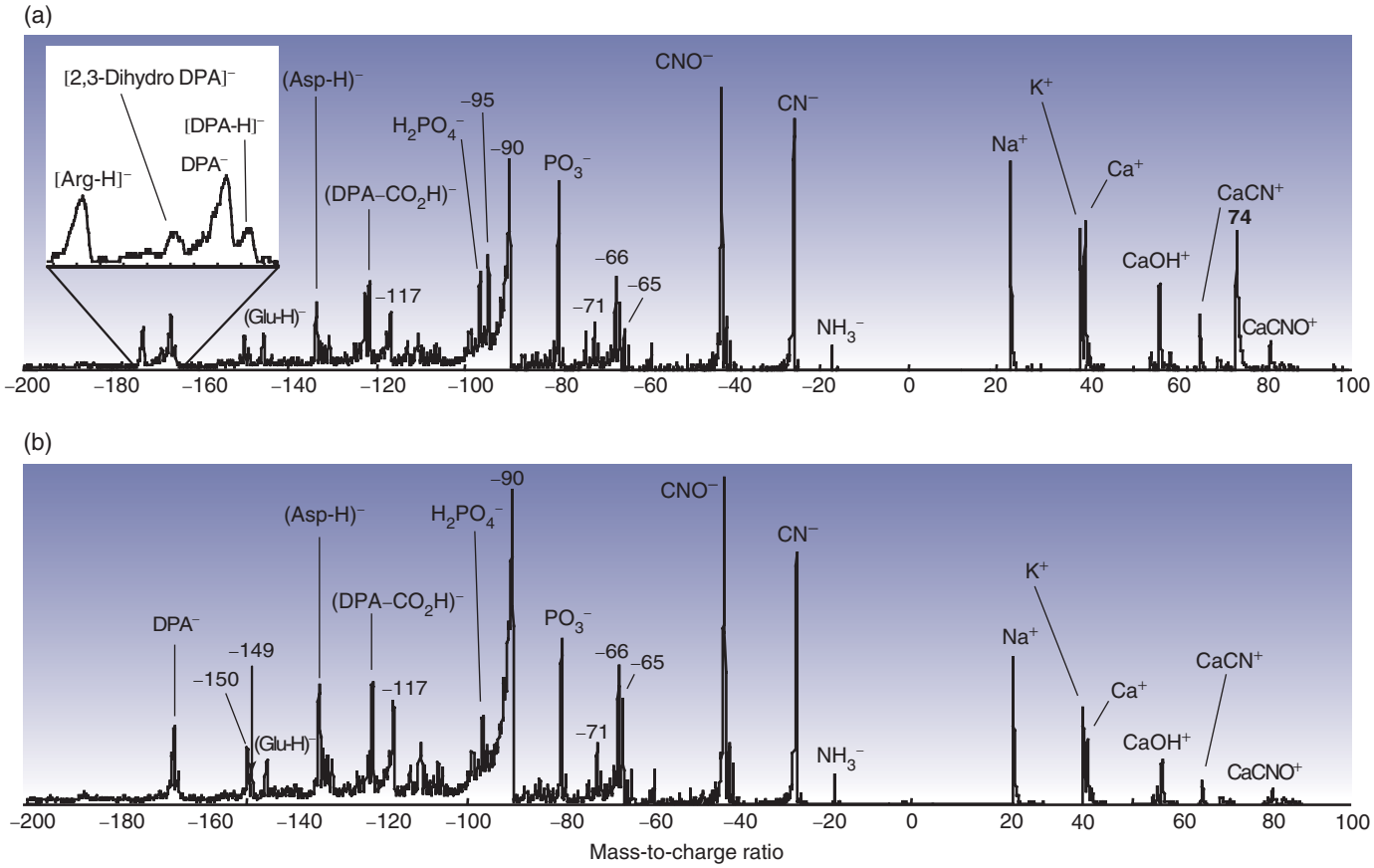


A schematic of a bioaerosol mass spectrometry (BAMS) system being used to analyze a bacterial spore. BAMS has the potential to identify bioagents, such as anthrax, from only a single spore or cell and to clarify the molecular changes that occur in normal and cancerous cells.

which is under a vacuum. While entering the vacuum, each particle accelerates to its own specific terminal velocity—a velocity that depends on a particle's size and shape but averages about 300 meters per second. The particles then pass, one at a time, through two continuous scattering laser beams, which are set serially in the path of the particles. Each particle scatters laser light as it passes through each beam. The time between the two scattering events provides information on a particle's velocity and size. Each particle continues on, zipping into the path of a third, pulsed ionization laser. The pulsed laser fires, desorbing and ionizing the particle and producing both negative and positive ions. The particle's journey—from entering the nozzle to annihilation by the ionizing beam—takes about 100 milliseconds. (See the figure at left.)

Spectra from these resulting ions are collected simultaneously by separate mass spectrometers. The spectra for each type of material are as unique as snowflakes. The spectra from one spore species differ in varying degrees from those of other *Bacillus* spores and are even more different from the spectra from a smoke particle, for instance. The spectra are first analyzed and categorized using real-time



Spectra of (a) *Bacillus subtilis var. niger* and (b) *Bacillus thuringiensis*, showing the peaks of greatest difference.

pattern recognition software developed at Livermore. Then, in a two-stage process, they are compared with spectra in a database of various substances gathered previously.

In the first stage, nonmicrobial (nonliving) particles such as smoke and flour are identified and removed from further analysis, while spectra of bacterial spores proceed to the next stage. In this second stage, the spectra of the bacterial spores are analyzed and classified by species. "A lot of data come in very quickly," says Gard. "We need to be accurate, first time out. For instance, a natural insecticide containing spores of *Bacillus* is similar in chemical structure to the anthrax pathogen. We need to be able to differentiate between them the first time, every time."

Tests Show the Difference

To test their system, the team used surrogates of anthrax (*Bacillus subtilis var. niger*) and a commonly used organic pesticide (*Bacillus thuringiensis*) that differs from *B. anthracis* in two short sections of its DNA. One technical reality the team had to work around is that the fast-traveling microorganism may encounter the ionizing laser beam at any point in the beam field. Because irregularities in the beam field exist—even in a beam of specific wavelength, pulse length, and fluence—this inhomogeneity results in the particle fragmenting into slightly different ions, depending on what part of the beam field it hits. The variation in the resulting ions makes it more difficult to identify the original material. Even so, subtle differences between the spectra of *B. subtilis var. niger* and *B. thuringiensis* can be detected. (See the lower figure at left.) In recent tests, the BAMS systems success rate was 93.2 percent.

A prototype of the system was taken to Florida in 2001 to help screen the overwhelming number of suspicious powders sent to the Florida Department of Health shortly after the anthrax exposures in the U.S. Postal Service. "The Department of Health was using methods that took three days to turn around a single sample. At the time, we wanted to see if we could do the analysis with our system in a few seconds," says Gard.

In earlier tests at a biosafety level 3 facility in Florida, the system detected *B. anthracis* spores from nonmicrobial background particles. These proof-of-principle experiments showed how *Bacillus* spores could be detected when mixed with biological and nonbiological materials. Some of the other materials included white powders, such as aspartame, medicated foot powder, gelatin, growth medium, baking soda, and powdered sugar as well as cigarette and wood smoke. In all these cases, the BAMS system was easily able to detect spores from all other materials.

The team is working on the next-generation system, with which they hope to improve the rate of detection by focusing on specific optical properties of particles of interest.

Identifying Cancer, Tracking Tuberculosis

The ideal method for identifying bioagents would be instantaneous and absolute. BAMS is heading in that direction.

The technique also holds great promise in the arena of public health. The team is in the final year of an LDRD project, headed by Matthias Frank and Eric Guard. The goal is to develop the BAMS technique primarily for biomedical applications, such as detecting cancer by analyzing individual cells in clinical biopsies or identifying the bacteria that cause tuberculosis.

"Some day, when the system is perfected for field use, BAMS could be smaller than a breadbox and detect particles in about a millisecond," says Gard. "A person would breathe into a mask. BAMS could sample the particles from the lungs and then identify and characterize the particles—instantaneously."

—Ann Parker

Key Words: anthrax, bioaerosol mass spectrometry (BAMS), bioterrorism, airborne pathogens.

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The bioaerosol mass spectrometry (BAMS) team includes (left to right) in the front row: Maurice Pitesky, Herb Tobias (aerosol science), Joanne Horn, David Ferguson (data analysis), and Eric Guard (team leader); middle row: Jim Birch, Vincent Riot, Matthias Frank (laser–particle interactions) and Bruce Woods; and back row: Paul Steele, Norm Madden, and Keith Coffee (next-generation system).